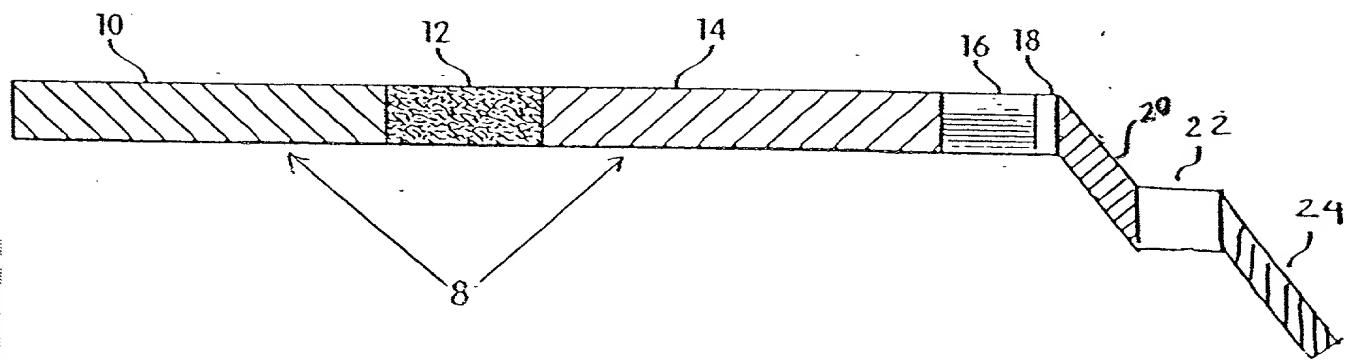
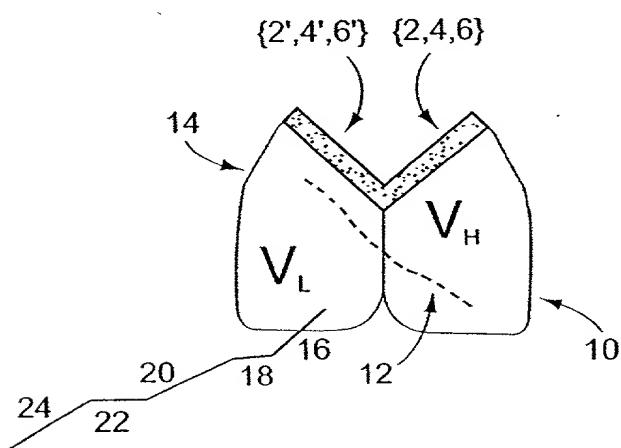


FIGURE 1

SINGLE CHAIN BINDING POLYPEPTIDE



(a) Extended Polypeptide



(b) Folded Protein

FIGURE 2

SINGLE CHAIN
BINDING POLYPEPTIDE SHOWING
LOCATIONS OF COMPLEMENTARITY
DETERMINING REGIONS, POLYPEPTIDE
SPACER REGIONS, AND EFFECTOR REGIONS

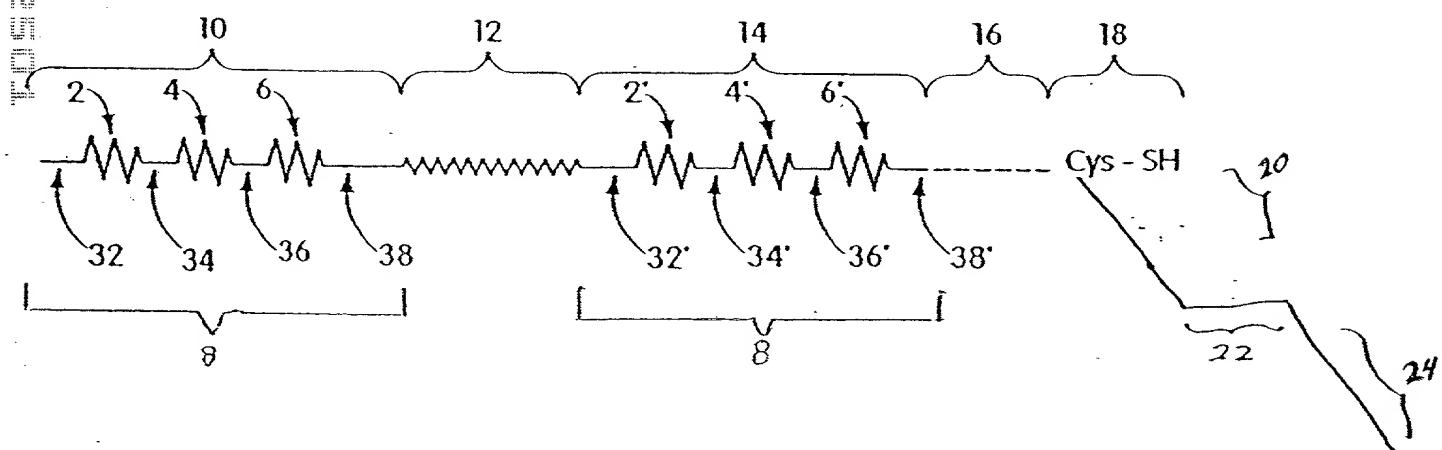


FIGURE 3

C6.5 sFv AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLLQSGAE LKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYML
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSLNCA KWPEYFQHWG QGTLTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCGSS SNIGNNYVSW YQQLPGTAPK
LLIYGHTRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCAAWDDSL
SGWVFGGGTK LTVLG

FIGURE 4

C6.5 sFv NUCLEOTIDE SEQUENCE

5' cagggtgcagctgtcagtcggggcagagttaaaaaaccggggagtcctgaagatctccgttaagggtctggataca
gctttaccagctactggatgcctggcgcagatgcccggaaaggcctggagttacatgggctcatctatctgggtactc
tgacaccaaatacagccgtccitccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcccactgcaatggagc
agtctgaagccctcgacagcgcgttatlttgcgcagacatgcgtggatattgcagtagttccaaactgcgcaaagtggcc
tgaataactccagcatggggccagggcacccctggtcaccgtcaccatcagggtggaggcgggtcaggcggaggtggctctggcg
gtggcggatcgcagtcgttgtacgcagccgcctcagtgctcggcccccaggacagaaggtcaccatcctcgtctggaa
gcagctccaaacatttggaaataattatgtatctggtaccagcagctccaggaaacagccccaaactccatctatggtcacacca
atcggcccgagggtccctgaccgattctcggctccaagtcggcaccctcagccctccatcagtgggtccgggtcccg
ggatgaggctgattattactgtcagcatggatgacagcctgagtggtgggttcggcggagggaccaagctgaccgtct
agg; 3'

FIGURE 5

C6ML3-9 sFv' AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGC-

FIGURE 6

C6ML3-9 sFv' NUCLEOTIDE SEQUENCE

5' caggtgcagctggcagtcggggcagaggtaaaaagccggggagtcctgaagatctccgttaagggtctggata
cagcttaccagctactggatgcctgggtgcgcagatgccggaaaggcctggagtcacatgggctcatctatcctg
gtgactctgacacccaaatacagccgccttcaaggccaggcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtcgttgaagccctcgacagcgcgtgtatgtgcgagacatgacgtggatattgcagtagttc
caactgcgcaaaagtggcctgaataacttccagcattgggccaggcaccctggtcaccgtctccctcaggtggaggcggtt
caggcggagggtggcttgcgggtggatgcgcgtctgtgttgcgcaggccctcagtcgtcggcccccaggacag
aaggcaccatctctgttgcgcgttgcgcgttgcgcaggccctcagtcgtcggcccccaggacag
agcccccaactccatctatgtatccatcaccatcgcccgagggtccctgaccgattctctggcttgcgcgttgcgc
cctcagccctccctggccatcagtgggtccggatgaggctgattactgtgcctccctggactacaccctc
tcgggtgggtttcggcggaggaaaccaagtcgcgccttaggtgcggccgcacaccatcatcaccatcacgggtgg
cggtgc 3'

FIGURE 7

C6ML3-9sFv'-L1-KDEL AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSEKD EL-

FIGURE 8

C6ML3-9 sFv'-L1-KDEL NUCLEOTIDE SEQUENCE

5' caggtgcagctggcagtcggcggcagaggtaaaaaagccggggagtctctgaagatctccgtaaagggtctggata
cagcttaccagctactggatgcgcctggcgccagatgcccggaaaggcctggaggatcatgggctcatctatcctg
gtgactctgacaccaaatacagccccgtcccaaggccaggcaccatctcgtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtctgaagccctcgacagcgccgtgtatttgtgcgagacatgacgtggatattcgttagttc
caactgcgcaagtgccctgaatacttccagcatggggccaggcaccctggtcaccgtctcctcagggtggaggcggtt
caggcggagggtggctctggcggtggcgatcgcagtcgtgtgcgacgcagcccccctcagggtctcggcccccaggacag
aaggtcaccatctctgctctggaaagcagctccaaacattggaaataattatgtatctggtaccagcagtcctccaggaaac
agcccccaactcctcatctatgtacacaccaatcgccccgcaggggtccctgaccgattctctggctccaagtctggca
cctcagccctccctggccatcagtgggtccggcaggatgaggctgattattactgtgcctccctggactacaccctc
tcgggtgggtgttcggcgaggaaaccaagctgaccgtcttaggtgcggccgcacaccatcatcaccatcacgggtgg
cggtccctcgagtctctggatccgaaaaaattatgtgcctccctggactacaccctc

FIGURE 9

C6ML3-9 sFv' -L2-KDEL AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSGSSSS GSEKDEL-

FIGURE 10

C6ML3-9sFv'-L2-KDEL NUCLEOTIDE SEQUENCE

5' caggtgeagctggtgcatctggggcagaggtaaaaaagcccgaaaaggctctgaagatctctgttaagggtctggata
cagctttaccagctactggatcgccctgggtgcgcaggatgcggggaaaggccctggaggatcatgggctcatctatcctg
gtgactctgacaccaaatacagccgtcctccaaggccaggatcaccatctcagtcgacaatccgtcagcactgcctac
ttgcaatggagcagtcgtgaagccctcgacagcgccgtgtatttgtgcgagacatgacgtggatattcagtagttc
caactgcgccaaagtggctgttgcataacttcagcatggggccaggccaccctggtcaccgtctccctcagggtggaggcggtt
caggccggagggtggctctggcggtggcgatcgcatgtgtgtgcgcaggccctcagggtctgcggcccccaggacag
aaggfcaccatctctgttgcataacttcagcatgttgcgcaggatcaccatccgttgcgcaggacatgttgcgcaggac
agcccccacactcctcatctatgtatcacaccaatcgcccgagggtccctgaccgattctctggctccaaatgttgcgc
cctcagcctccctggccatcagtgggtccggatcgaggatgaggatgttgcgcctctggactacaccctc
tcgggctgggtgtcggcgaggaaaccaagctgaccgtccctagggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgcctcgagtcatagcagctccggttccctctagc tctggatccg aaaaagatga actg 3'

FIGURE 11

C6ML3-9 sFv'-L2-H14 AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLTVVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSSSS
GSKKSAKKTP KKAKKP-

FIGURE 12

C6ML3-9 sFv' -L2-H14 NUCLEOTIDE SEQUENCE

5' caggtgcagctggcagtcggcagggcagaggtaaaaaagccggggagtcgtcgaagatctccgtaaagggttcgtgata
cagcttaccagctactggatcgctgggtgcgccagatgccggaaaggccgtggagtcacatgggcgtcatctatcctg
gtgactctgacaccaaatacagccgtccccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
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caactgcgcaaaatggccgtaaatactccagcattggccaggcaccctggtcaccgtcctccatagggtggaggcgggtt
caggcggagggtggctctggcggtggatcgcaaggctgtgtgtgcgcaggccctcagtgtcgccggccaggacag
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cctcagcccccgtggccatcagtgggtccggatggatgaggctgattattactgtccctctggactacaccctc
tcgggctgggttcggcgaggaaaccatcgccgtccctgaccgttcgtccaaagtctggca
cggctgc ctcgagtcta gcagtcgg ttccatcgtc tctggatcca agaaaagcgc gaaaaagacc ccgaagaaag
cgaagaaacc g 3'

FIGURE 13

C6ML3-9sFv'-L2-nls AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSSSS
GSTPPKKKRK V

FIGURE 14

C6ML3-9 sFv'-L2-nls NUCLEOTIDE SEQUENCE

5' caggtgeagctggcagtcggggcagaggtgaaaaagcccgaaaaggctctgaagatctccgtaaagggtctggata
cagcttaccagactacggatcgccctggcgccagatgcccggaaaggccctggagtcacatggggctcatctatccctg
gtgactctgacaccaaatacagccgtccccaaggccaggtcaccatctcagtcacaagtccgtcagcactgcctac
ttgcaatggagcagtcgtaaagccctggacagcgccgttatggcgagacatgacgtggatattgcagtagttc
caactgcgcacaagtggcctgaataacttccagcattgggcccaggccacccgttgcgcggatattgcagtagttc
caggcggagggtggcttgcgggtggcgatcgcaactgtgttgcgcggatattgcagtagttc
aaggfcaccatctctgttgcggatcgcaacttccatctatgttgcgcggatattgcagtagttc
aaggtcccaacttccatctatgttgcgcggatattgcgcggatattgcagtagttc
cctcagccctccctggccatcagtgggtcccgaggatgaggctgattattactgtgcctctggactacaccctc
tcggcgtgggtttcggcgaggaaaccaagctgaccgtccatgtgcggccgcacaccatcatcaccatcacgggtgg
cggtgc ctggatcca gcagctccgg ttcccttagc tctggatcca ctccgcccggaa aaaaacgt aaagt 3'

Figure 15. C6ML3-9 sFv' and its salmon protamine conjugate binds specifically to the erbB-2 positive ovarian cancer cells

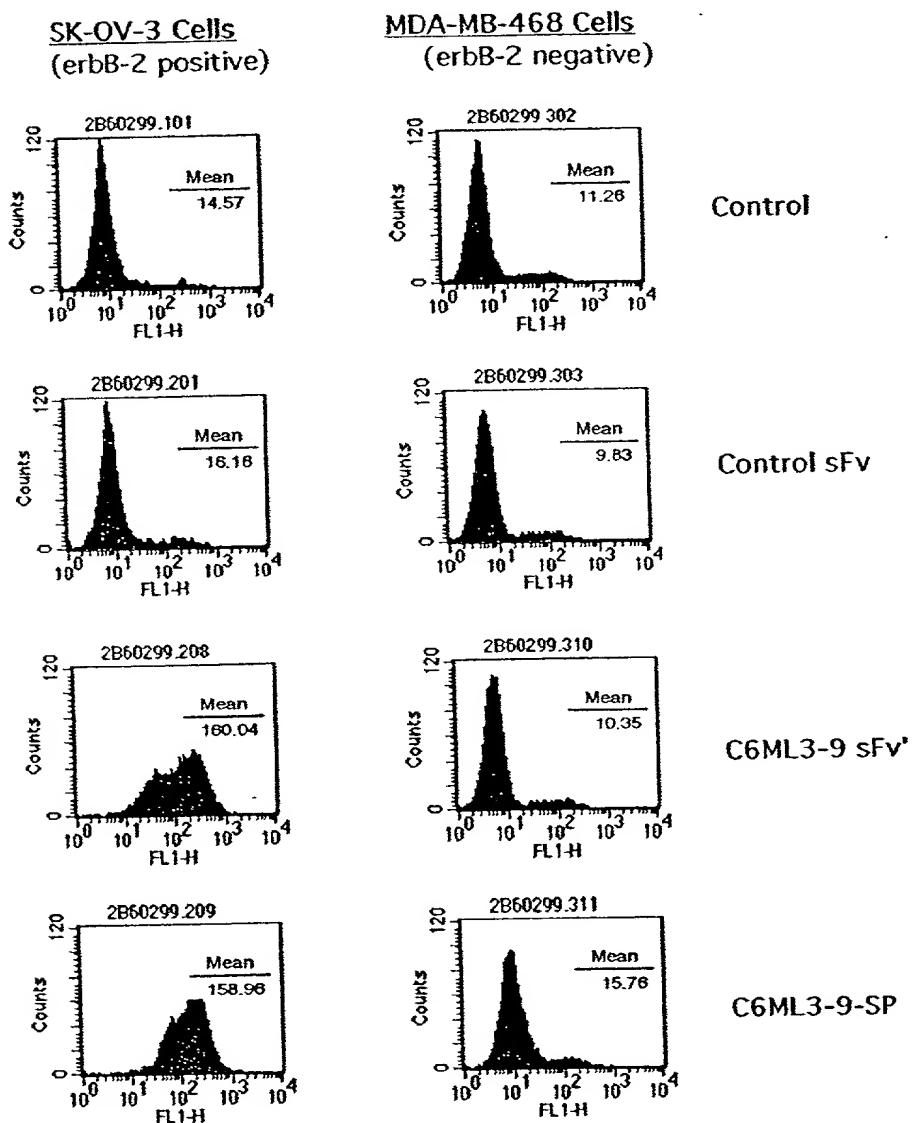


Figure 16. FACS Analysis of the erbB-2 Binding Activities of Bacterially Expressed C6ML3-9 sFv' and its Derivatives

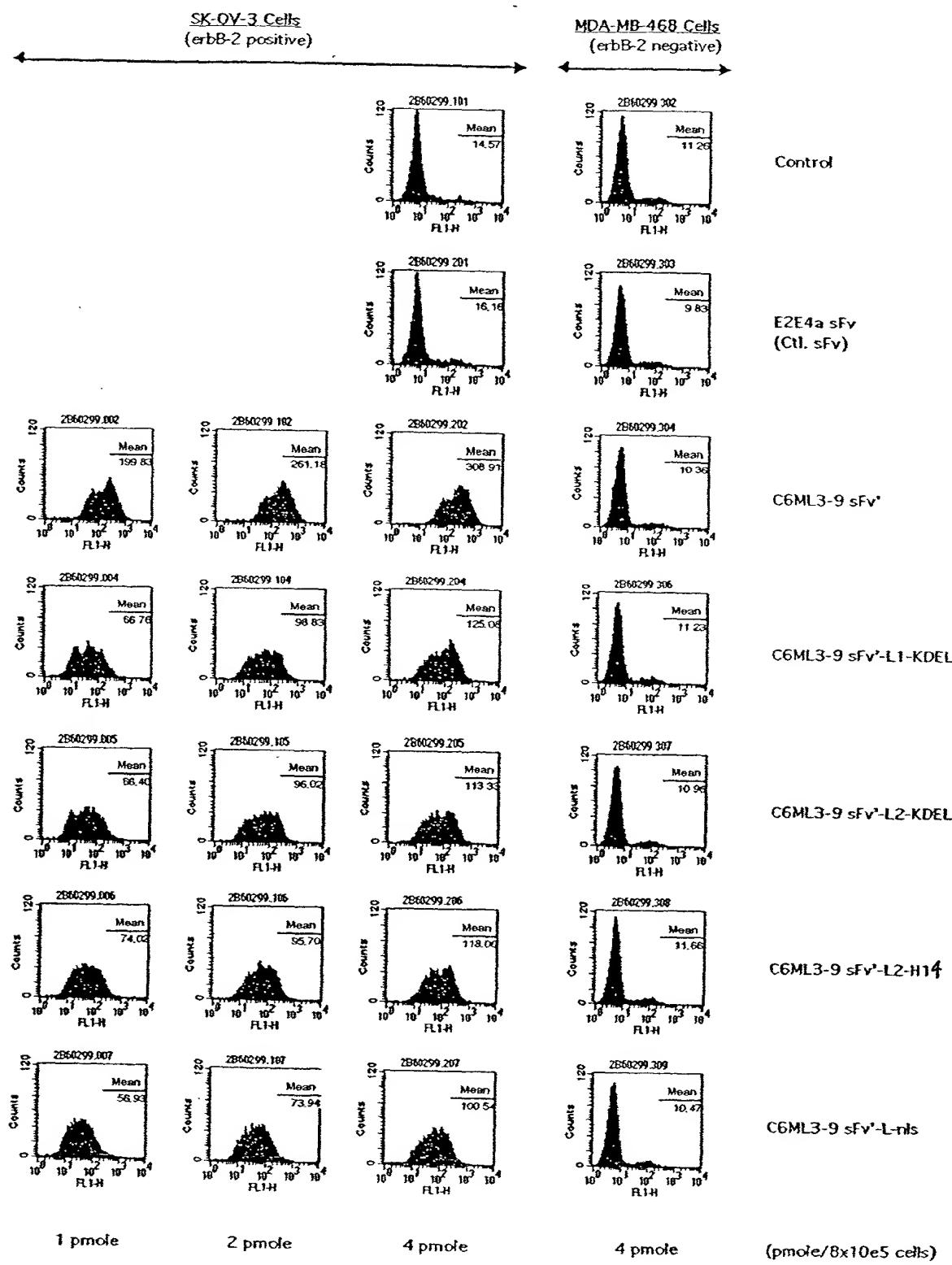
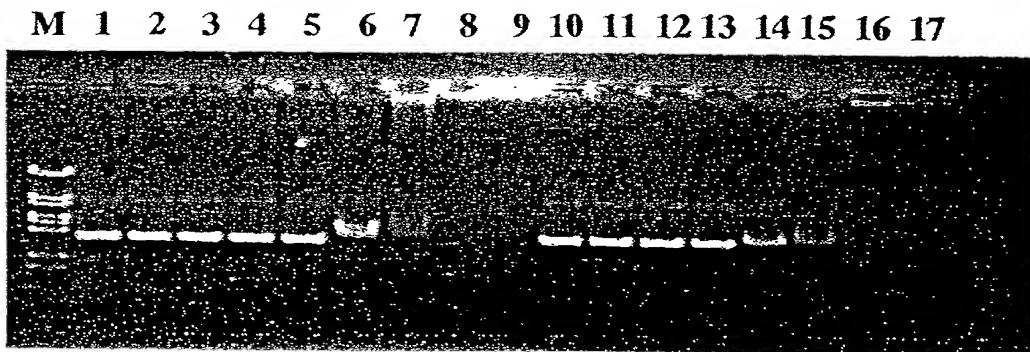


Figure 17. Gel Shift Analysis of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex

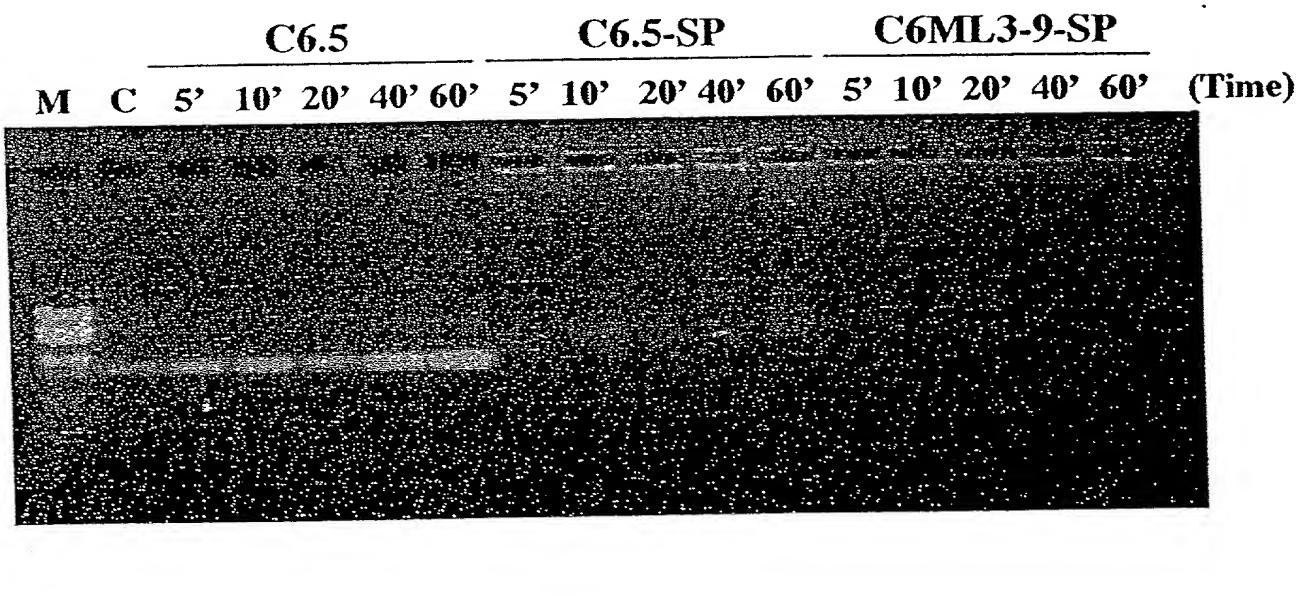


M. DNA marker - λ DNA BstEII digest

1. 200 ng pGL3 DNA
2. 200 ng pGL3 DNA + 1.45 pmol C6.5
3. 200 ng pGL3 DNA + 2.90 pmol C6.5
4. 200 ng pGL3 DNA + 5.80 pmol C6.5
5. 200 ng pGL3 DNA + 11.6 pmol C6.5
6. 200 ng pGL3 DNA + 1.45 pmol C6.5-SP
7. 200 ng pGL3 DNA + 2.90 pmol C6.5-SP
8. 200 ng pGL3 DNA + 5.80 pmol C6.5-SP
9. 200 ng pGL3 DNA + 11.6 pmol C6.5-SP
10. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9
11. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9
12. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9
13. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9
14. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9-SP
15. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9-SP
16. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9-SP
17. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9-SP

*0.8% agarose gel in 1xTAE, 150v, RT, ~1hr, EtBr staining overnight

Figure 18. Kinetic Study of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex Formation



M. DNA marker - λ DNA BstEII digest

C. 200 ng pGL3 DNA alone

* The rest of the lanes - 200 ng pGL3 DNA incubated with 5.8 pmol proteins as indicated above each line, on ice, for different period of time. Electrophoresis condition same as Figure 3.

Figure 19. The C6ML3-9-SP conjugate protein mediates specific luciferase gene delivery to erbB-2 positive cancer cells

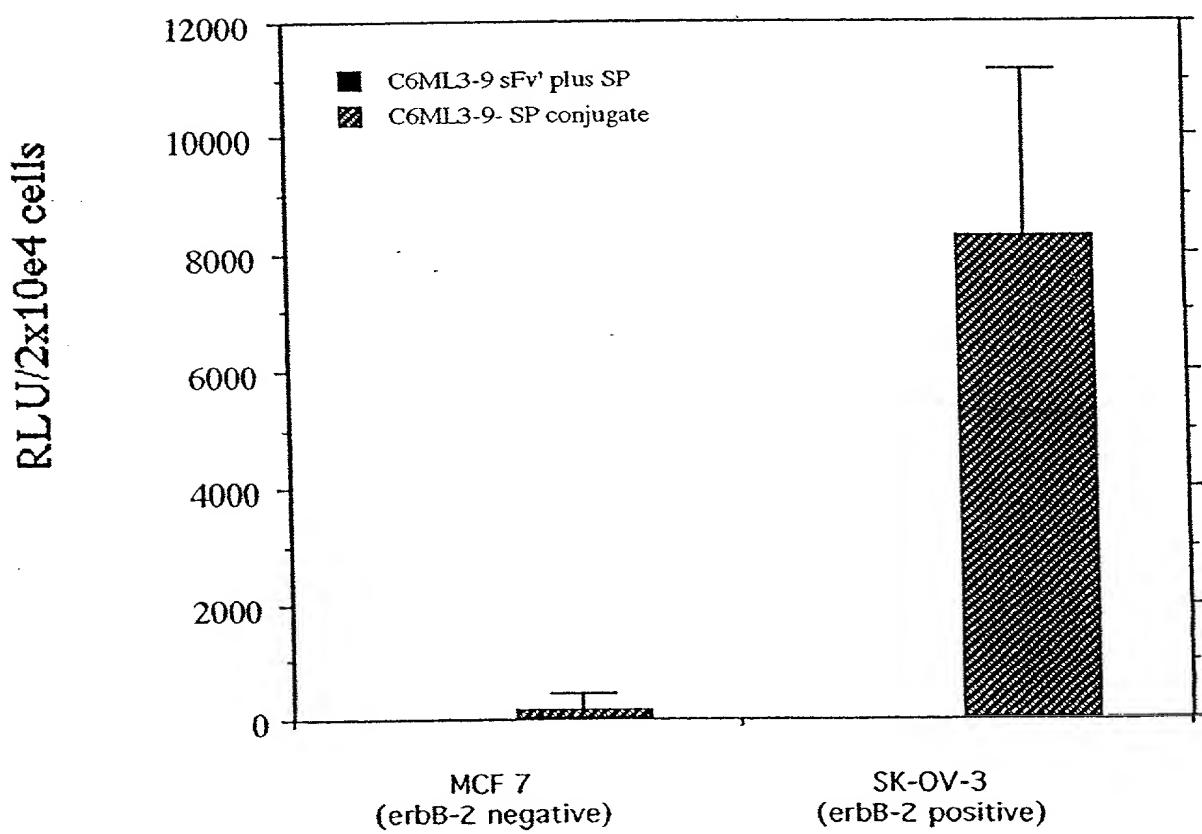


Figure 20. Chloroquine-dependent C6ML3-9-SP-mediated Gene Delivery

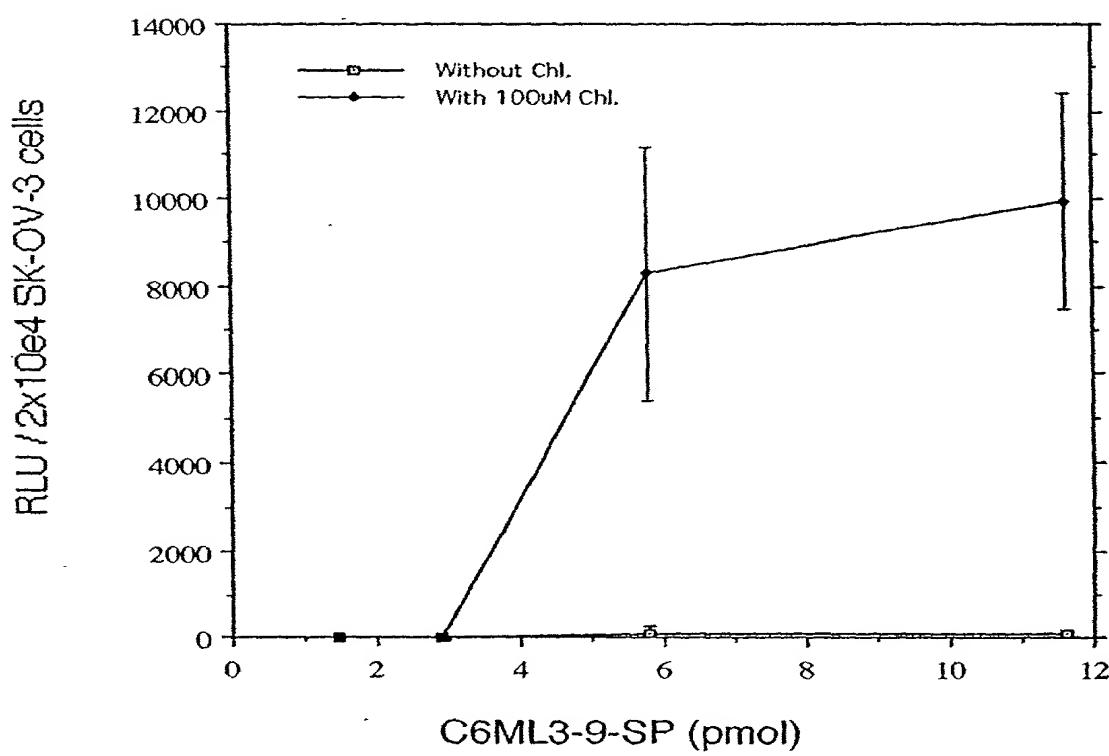


Figure 21. Fluorescent microscopy of C6.5-SP and C6ML3-9-SP-mediated gene transfer of pGeneGrip Rhodamine/GFP plasmids with SK-OV-3 and MCF-7

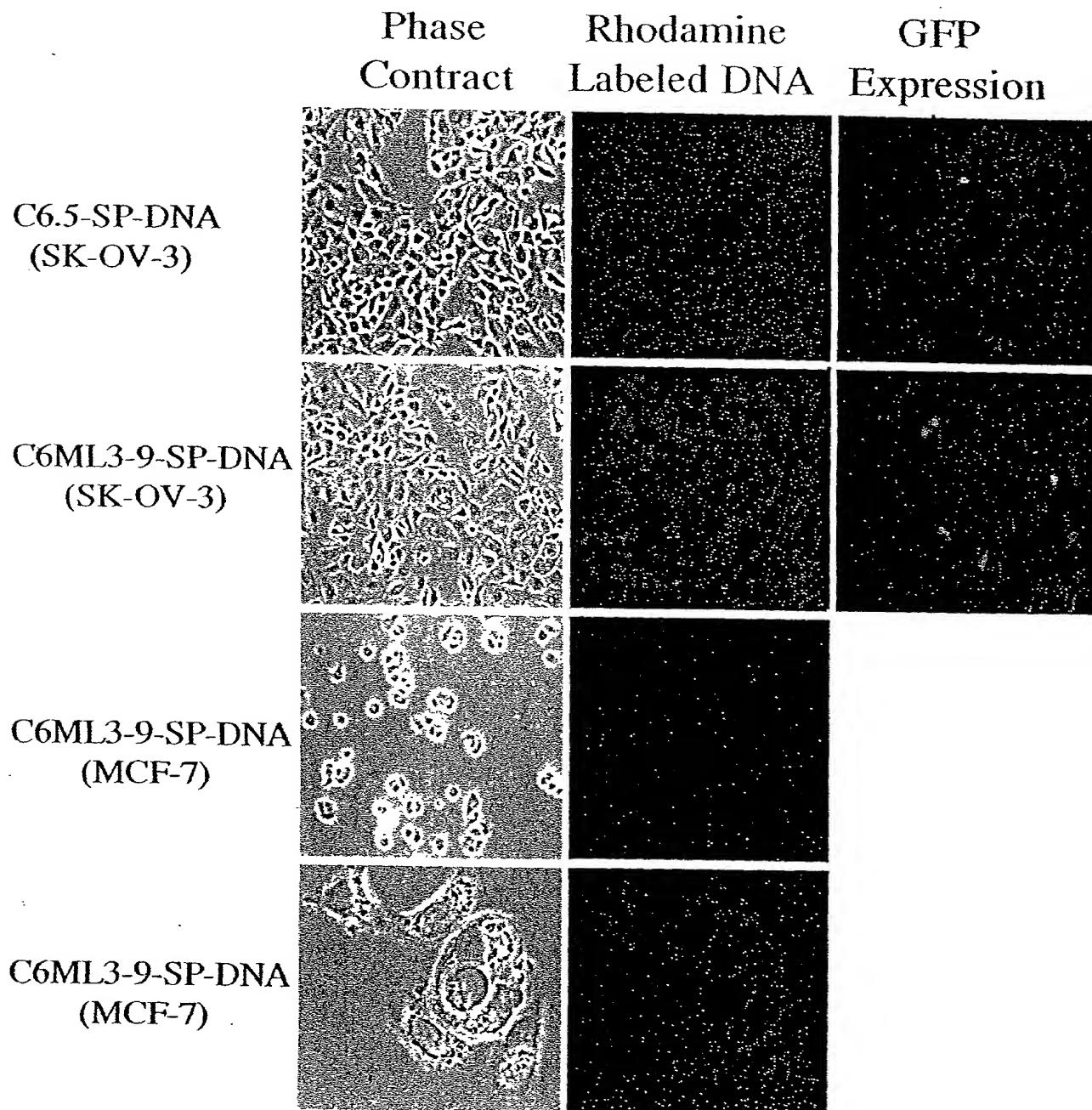


FIGURE 22

THE EFFECT OF CHLOROQUINE ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'-SALMON PROTAMINE

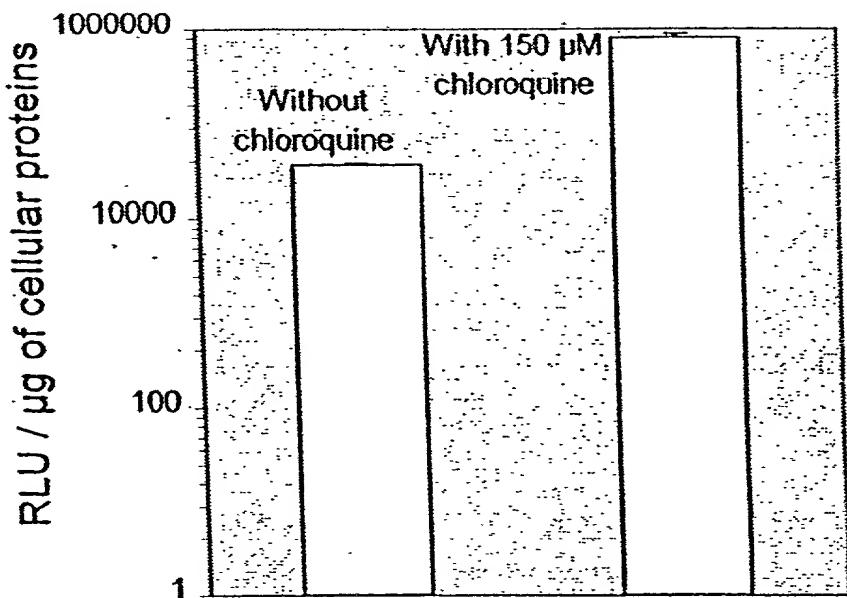


FIGURE 23

THE EFFECT OF CHLOROQUINE ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-P1

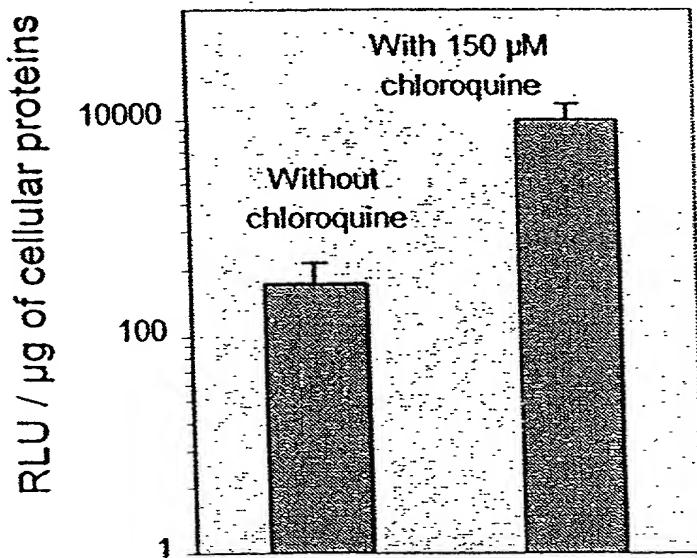


FIGURE 24

THE EFFECT OF CHLOROQUINE ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-H1

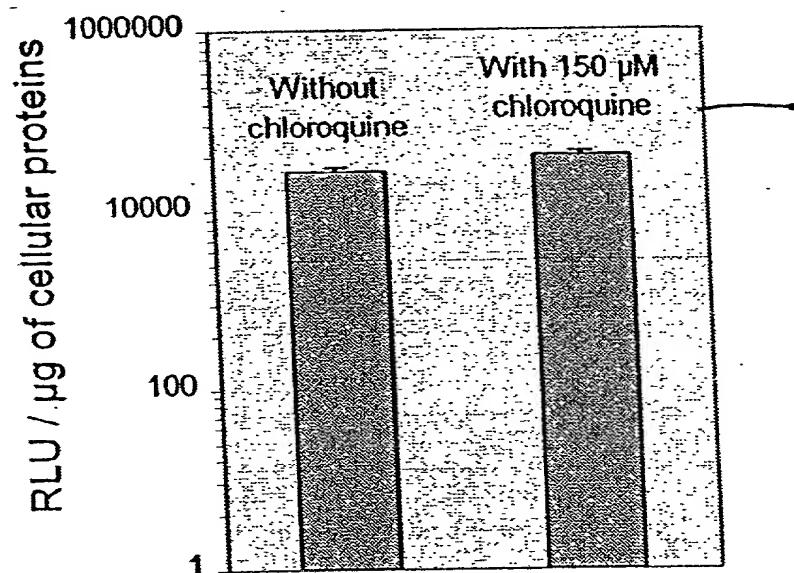


FIGURE 25

THE EFFECT OF C6ML3-9sFv'-H1-pBks ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'-H1

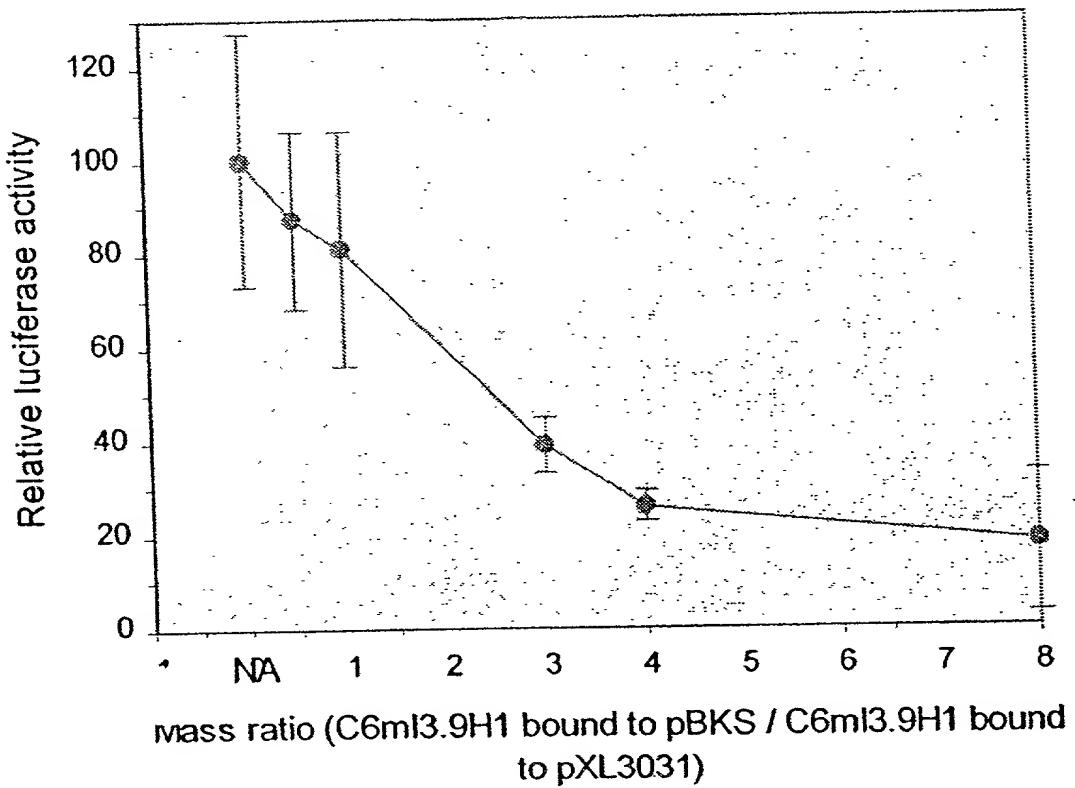


FIGURE 26

THE EFFECT OF THE DNA TO C6ML3-9sFv'-H1 RATIO ON 3T3-HER2 TRANSFECTION EFFICIENCY

